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Effects of Temperature and Redox Conditions on Degradation of Chlorinated Phenols in Freshwater Sediments

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EFFECTS OF TEMPERATURE AND REDOX CONDITIONS ON DEGRADATION OF CHLORINATED PHENOLS IN FRESHWATER SEDIMENTS

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FOREWORD

As environmental controls become more expensive and penalties for judgment errors become more severe, environmental management requires more precise assessment tools based on greater knowledge of relevant phenomena. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Biology Branch conducts research to predict the rate, extent, and products of biological processes that control pollutant fate in soil and water and develops methods for forecasting ecosystem level effects suitable for exposure and risk assessment.

Mathematical models that predict the transport and transformation of toxic compounds in the environment rely on accurate input data about the physical and chemical properties that affect chemical fate. Among the properties that influence chemical as well as microbiological fate processes are temperature and oxidation-reduction conditions. This report examines the effects of these two properties on the dechlorination of two potentially hazardous compounds, 2,4-dichlorophenol and 4-chlorophenol, in sediment slurries.

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ABSTRACT

The effect of temperature and redox conditions on the anaerobic degradation of 2,4-dichlorophenol (2,4-DCP) was investigated in anaerobic sediment slurries, prepared from local freshwater pond sediments. Under methanogenic conditions, 2,4-DCP dechlorination occurred in the temperature range between 5 and 50°C. Although dechlorination was not observed above 50°C, anaerobic bacterial activity was indicated by methane formation up to 60°C. In sediment samples from two sites and at all temperatures from 5°C to 50°C, 2,4-DCP was transformed to 4-chlorophenol (4-CP). The 4-CP intermediate was subsequently degraded after an extended lag period. Adaptation periods for 2,4-DCP transformation decreased between 5°C and 25°C, were essentially constant 25°C and 35°C, and increased between 35°C and 40°C. Degradation rates increased exponentially between 15°C and 30°C, had a second peak at 35°C, and decreased to about 5% of the peak activity by 40°C. In one sediment sample, an increase in degradation rates was observed following the minimum at 40°C, suggesting that at least two different organisms were involved in the 2,4-DCP dechlorination.

The addition of sulfate at a level of 0.25% resulted in a marked reduction in the rate of methane formation, a marked reduction in the rate of reductive dechlorination, and an increase in the length of the adaptation period before reductive dechlorination could be detected. The addition of nitrate at a level of 0.15% resulted in the complete inhibition of the reductive dechlorination reaction for at least 127 days.

Storage of the original sediment slurries for 2 months at 12°C resulted in increased adaptation times, but did not affect the degradation rates.

This report covers a period from October 21, 1985, to June 30, 1988, and work was completed as of May 30, 1988.

INTRODUCTION

The physical and chemical properties of the sediment environment can vary in time as well as in location. Sediment temperatures change over the seasons, and sediments directly exposed to the sun in summer can reach temperatures in excess of 50°C. Freshwater sediments are primarily methanogenic and marine sediments are dominated by sulfate reducing conditions. These fluctuations and differences can influence chemical as well as microbiological processes leading to the transformation or degradation of hazardous organic chemicals. Therefore, during the development of mathematical models to predict the fate of toxic compounds in the environment, it is important to include parameters that describe this influence of temperature and chemical characteristics on anaerobic biodegradation.

We report here the effect of temperature and redox conditions on the reductive dechlorination of 2,4-dichlorophenol (2,4-DCP) and 4-chlorophenol (4-CP) in sediment slurries. We also show that, for a restricted temperature range (e.g. from 15°C to 30°C), dechlorination rates under methanogenic conditions can be described by an Arrhenius function.

Both 2,4-DCP and 4-CP are released into the environment by direct disposal or as break down products of higher chlorinated compounds (5,10) such as the extensively used wood preservative pentachlorophenol (PCP) or the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). They are highly toxic and 4-chlorophenol is a suspected carcinogen (12). Thus, there is a clear need for modeling the fate of these compounds in order to evaluate their potential risk to man and his environment.

The reductive dechlorination of chlorophenols has been reported in a variety of anaerobic sediments such as sewage sludge (1,2), sediment (4), and subsurface aquifer environments (4), all of which were methanogenic. Only recently has the reductive dechlorination of 2,4-dichlorophenol been reported to occur under sulfate reducing conditions (8), and to our knowledge, reductive dechlorination under denitrifying conditions has not been reported. The proposed pathway for the anaerobic degradation of 2,4-DCP is the progressive replacement of the chlorines by hydrogen (reductive dechlorination) leading to phenol, which is then further degraded to methane and carbon dioxide (6,7). Similar reductive pathways have been reported for chlorinated benzoates (11,13,16,18,19,21) and other chlorinated phenols (1,2,3,6,17).

Gibson and Suflita (4) observed that the addition of sulfate (29 mM) to methanogenic aquifer materials resulted in the inhibition of the dechlorination reaction. Interestingly, they also observed that the degradation intermediate phenol was readily degraded under sulfate reducing conditions. Smolenski and Suflita (14) found that adding sulfate (20 mM) to methanogenic aquifer material led to increased degradation of cresols. Unfortunately, little else is known about the effects of environmental factors on these reactions. An extensive study of the effects of redox couples or temperature has not been reported. Our work was undertaken to learn more of these effects to provide a better base for the development of environmental fate and effects models.

MATERIALS AND METHODS

Sediments. Freshwater lake sediments from two different sites near Athens, GA, were used in this investigation. One set of samples was collected (June 1986) from a pond at the Sandy Creek Nature Center (SCNC), which is located in a wooded area. The Nature Center site was established twelve years ago, and since then all direct discharge of pollutants into the pond has been prohibited. The other sediment was collected (June 1987) from a small lake close to the Cherokee Trailer Park (CTP). This lake is bordered on one side by the trailer park and by farm land on the other. Samples were taken near a new asphalt road on the west side of the lake. These sediments were found to contain small rocks coated with asphalt. In each case, the upper 5 to 8 cm of the sediment were collected in 1.5 L glass jars. The jars were immediately capped with a butyl rubber stopper, brought to the laboratory and purged for 1 hour with oxygen-free nitrogen. The sediments were either used immediately or after storage at 12°C in a refrigerator. Before sediment collection, the pH, temperature and redox potential conditions at each sampling site were determined (Digi-Sence pH-meter with pH electrode and temperature probe, Cole Palmer Instr. Co., and platinum/Ag/AgCl combination redox electrode from Fisher Scientific Co.). Total organic carbon was determined with a carbon analyzer (model 524C carbon analyzer, O. I. Corporation).

2,4-Dichlorophenol transformation experiments. Individual reaction flasks consisted of anaerobic tubes that were filled with 20 ml of sediment slurry and crimp sealed with butyl rubber stoppers (Bellco Co.). All procedures were conducted under flowing nitrogen that had been purged of oxygen. Methanogenic slurries were prepared by combining sediment with site water to give 0.2 to 0.4 mg dry wt. sediment/ml of slurry, adding sufficient 1 M K_2HPO4/KH_2PO_4 to yield a buffer concentration of 20 mM, and then adjusting the pH to 6.9 by the addition of small quantities of 2 N NaOH. The slurries were prepared in a blender (Hamilton Beach Co.) and were periodically mixed to obtain a homogeneous solution. Sulfate reducing or denitrifying slurries were prepared by adding sulfate (0.25%) or nitrate (0.15%), respectively.

Degradation experiments were initiated by adding 0.7 ml of an aqueous 2,4-DCP stock solution to give a final concentration of about 125 µmol/l (20 ppm). Duplicate samples were incubated in waterbaths (modified dry-baths by Thermolyne Co. to ensure that the contents of the tubes were completely submerged) at different temperatures. Temperatures below ambient were obtained by placing the waterbaths in refrigerators. Sediment slurries were removed periodically and combined with an equal volume of acetonitrile to terminate the reaction and to facilitate recovery of compounds bound to the sediment matrix. These mixtures were subsequently analyzed by high performance liquid chromatography (HPLC) for 2,4-DCP, 4-CP, phenol, and benzoate. Abiotic controls were prepared by autoclaving the sediment samples on three consecutive days for 45 min each before 2,4-DCP was added.

Methane formation experiments. For methane formation experiments, 10 ml of the slurry without 2,4-DCP were placed in Hungate tubes and the filled tubes were then incubated in a temperature gradient incubator (7 to 80°C) with continuous shaking (model 675, Scientific Industries Inc.). Periodically,

100-ul samples were taken from the headspace with a gas lock syringe (Dynatech Precision Sampling Corp.) and the methane concentration determined by gas chromatography.

Analytical procedures. Sediment slurry samples (0.25 ml) were mixed with an equal volume of acetonitrile, shaken for 3 min (Vortex-Genie 2 with a sample head for micro test tubes, Scientific Industries, Inc.) and then centrifuged at 16,000 x g for 10 min (Microfuge E, Beckman Instr. Inc.). The supernatant solution was analyzed for chlorophenols and phenols using an HPLC-system (Rainin Instr. Co.) with a C-18 reversed phase column (Dynamax Microsorb Short One with guard column, Rainin Instr. Co.). The mobile phase consisted of methanol, water and acetic acid (60:38:2 v/v). UV absorption was monitored at 285 nm and 2,4-DCP and 4-CP concentrations were determined with a calibrated integrator (CR-3A, Shimadzu Scientific Instr. Inc.). Gas headspace samples from methane formation experiments were analyzed with a gas chromatograph (Vista 44 with Data System Vista 401, Varian Associates, Inc.) using a Chromesorb column (10% Sp1000, 1% H_3PO_4, Supelco Inc.) at 60°C and an FID detector. Total organic carbon was determined by using a carbon analyzer (model 524C carbon analyzer, O. I. Corporation) following the procedure described in the manual. Soluble sulfate levels were determined with a Dionex 2020I ion chromatograph. A fast run anion column was used with a 0,003 M $KHCO_3$/0.0024 M K_2CO_3 eluent run at 2.5 ml/minute, and a 0.025 N H_2SO_4 solution was used with the continuous flow fiber suppresser.

Chemicals. All phenolic compounds were purchased from Aldrich Chemical Co. Inc. (highest purity available) and other chemicals and solvents (HPLC grade) were from J. T. Baker Chemical Co.

Calculation of degradation parameters. All data, except the dechlorination data between 43°C and 50°C, represent the average of duplicate experiments. In all experiments, a lag period was observed before a loss of 2,4-DCP occurred and before the appearance of 4-CP could be detected. We refer to this time period as the adaptation period. The adaptation period for 4-CP was defined as the time period between the first appearance of 4-CP and the first significant detectable loss of this compound. Rates of 2,4-DCP and 4-CP loss were determined by measuring the maximal slope in the substrate concentration versus time plots that had been first corrected for abiotic losses in autoclaved controls. The rates of methane formation were determined by dividing the accumulated methane concentration by the total number of days of incubation. The Arrhenius energy (EA) was determined from a modified Arrhenius plot. The log10 of the loss rate was plotted versus the reciprocal temperature (K) and the EA was calculated from the slope of the linear portion of the curve.

RESULTS

Sediment characterization. Sediment samples were collected in June 1986 from the SCNC pond (SCNC) and in June 1987 from the CTP pond. The conditions of the sites are summarized in Table 1. Sediments at both sites were dominated by methanogenic communities. To minimize artifacts due to sample preparation, no reducing agent was added. Periodic measurements of methane formation during degradation experiments were monitored to ensure anaerobic conditions to the level of methanogenesis.

Methane formation. The rates of methane formation versus temperature profiles of both sites are shown in Figure 1a. Two major peaks of methane production were observed with both sediments. The first peak occurred between 28 and 40°C and the second occurred between 50 and 58°C. A pronounced minimum was observed around 44 to 48°C. No methane formation was detectable above 60°C. Some significant differences were observed, however, in the shape of the first peak. In the sediment slurry from the CTP site, a broad peak with a maximum at 35°C was observed, whereas, several reproducible smaller peaks at 30, 35, and 43°C with intermediate minima at 33 and 40°C were observed with the sediment from the SCNC site.

The addition of sulfate to the sediment slurries resulted in a conspicuous decrease in the rate of methane formation (Fig 1b). Interestingly, the major peak of activity observed at 43°C in the presence of sulfate matched well with a similar peak of activity observed in the absence of sulfate. No methanogenic activity was observed in slurries amended with nitrate (0.15%).

Dechlorination of 2,4-dichlorophenol. In both sediments, anaerobic biodegradation of 2,4-DCP occurred in the temperature range between 5 and 40°C and good correlations between replicates were observed. Above 40°C, however, biodegradation activity was intermittent and only half of the replicates at each temperature of the stored SCNC sediment were active up to 52°C, whereas for the fresh sediment, only a single replicate at 43°C was active above 40°C. When dechlorination was observed, 2,4-DCP was transformed stoichiometrically to 4-CP (Fig. 2a) after an initial adaptation period. The 4-CP product was subsequently degraded following a second, more lengthy adaptation period. Phenol, which was detectable down to 10 µmol/l (about 5% of the initial dichlorophenol concentration), did not appear as an intermediate product in these experiments.

In the presence of sulfate (0.25%), the reductive dechlorination of 2,4-DCP to 4-CP was markedly reduced and no detectable loss of the 4-CP product was observed after 365 days of incubation. No dechlorination of 2,4-DCP was observed for a period of 127 days when nitrate (0.15%) was added to the sediment slurries.

Abiotic losses of 2,4-DCP were quantified in autoclaved controls. Recovery was dependent on the temperature and the length of incubation (Fig. 3). For example, no significant loss of parent compound was observed at 11°C over an incubation period of 270 days. At higher temperatures, increased losses of 2,4-DCP were observed, and at 72°C, only 15% of the initial amount was present after 100 days. In all autoclaved samples, only the disappearance of the parent compound occurred. The formation of 4-CP, phenol or benzoate was not

observed. The disappearance of 2,4-DCP is assumed to result from higher concentrations of the compound in the gas phase at higher temperature and, consequently, a partial absorption in the butyl rubber stopper.

Adaptation periods. The effect of temperature on the adaptation period for 2,4-DCP transformation was similar for the two fresh sediments over the range from 5°C to 40°C (Fig. 4a). Between 25 and 35°C the adaptation periods, were similar with values ranging from 7 to 11 days. Below 25°C, the adaptation periods increased with decreasing temperature, and between 35 and 40°C, they increased with increasing temperature. Between 40 and 52°C, no dechlorination activity was observed with the CTP sediment and only one replicate at 43°C was active for the SCNC sediment. Interestingly, a marked shortening of the adaptation period was observed at 43°C for active SCNC sediments.

When stored SCNC sediments were investigated, the adaptation periods were similar to fresh sediments from 25 to 35°C; however, below 25°C and above 35°C the adaptation periods were found to be about twice those observed for the fresh sediments. Above 43°C, an increase in the adaptation period was again observed as the temperature was raised to 52°C. Only one replicate was active at each of the higher temperatures, however.

Adaptation periods for 4-CP were longer at all temperatures (Fig. 4b) compared to adaptation periods for 2,4-DCP transformation. The 4-CP adaptation periods were at a minimum between 30 and 35°C and increased below and above this temperature range. A two-fold increase in adaptation time was observed with stored SCNC sediments. Apparently, storage had a similar effect on the adaptation periods for both 2,4-DCP and 4-CP.

Adaptation periods under sulfate reducing conditions were generally longer (Fig. 4c) for the dechlorination of 2,4-DCP. The increase ranged from 460% at 18°C to as little as 8% at 40°C. No detectable loss of 2,4-DCP was observed for slurries incubated at temperatures below 18°C and above 40°C and no detectable loss of the 4-CP was observed at all temperatures.

Transformation rates. Rates of 2,4-DCP transformation reproducibly increased in the CTP , fresh SCNC (not shown) and stored SCNC sediments between 5°C and 30°C, decreased to an intermediate minimum at 33°C, increased to a second peak at 35°C, and then decreased to about 5% of the peak activity by 40°C (Fig. 5a). A third peak of activity was observed between 40 and 52°C for the SCNC sediments; however, only half of the replicates at each temperature were active for the stored SCNC sediment and only the one replicated at 43°C was active for the fresh SCNC sediment. Unlike the adaptation periods, no effect of storage was observed on the transformation rates.

The 4-CP transformation rates were much slower by comparison. The 4-CP rates averaged about 10% of the 2,4-DCP rates over the temperature range between 15 and 40°C (Fig. 5a). Over an incubation period of one year (SCNC sediment) or 220 days (CTP sediment), no 4-CP was degraded below 15°C or above 40°C.

For SCNC sediments, a significant similarity occurred between the rate maxima of 2,4-DCP transformation and methane formation over the temperature range from 5 to 45°C (Fig. 5b). Both activities had maxima at 30, 35 and 43°C, and minima at 33 and 40°C. A similar correlation was not observed for the CTP sediment, although the transformation rates were similar to those of the SCNC sediment (compare Fig. 1 and Fig. 5a).

Under sulfate reducing conditions, the rate of reductive dechlorination activity was observed only (Fig. 5c) between 18 and 40°C after 365 days of incubation. Greater than 30% of the sulfate remained following complete degradation of the 2,4-DCP and methanogenic activity was inhibited for the duration of the experiment. The rates varied from 2 to 19% of the rates observed under methanogenic conditions. No reductive dechlorination was observed under denitrifying conditions after 127 days of incubation.

Arrhenius plots of the transformation rate data were linear between 15 and 30°C, indicating an exponential increase in the rates over the temperature range (Fig. 6). The activation energy EA, derived from the slopes of the linear portions, was 21 kcal/mol for methane formation and 13 kcal/mol for the 2,4-DCP transformation with the SCNC sediment, and 26 kcal/mol and 17 kcal/mol for the CTP sediment.

DISCUSSION

In our experiments, we observed a sequential dechlorination of 2,4-DCP in the temperature range between 5 and 52°C for unacclimated sediment dominated by methanogenic activity. At all temperatures, the first product was 4-CP, and although at least two different groups of microorganisms were involved in 2,4-DCP degradation at the different temperatures, they all used the same reaction to initiate degradation.

A similar reductive pathway has been reported by Gibson and Suflita (4) for the degradation of 2,4-DCP in anaerobic sediments at room temperature and by Boyd and Shelton (1,2) in sewage sludge at 35 to 37°C. Boyd and Shelton (1) found that 90% of ^{14}C-labeled 2,4-DCP and 4-CP was degraded to $^{14}CO_2$ and $^{14}CH_4$ in unacclimated sludge. They also observed in studies with monochlorophenols that chlorine in the 2-position was the most readily lost and that chlorine in the 4-position was the most stable. Interesting, we observed somewhat similar results in that the 2-chlorine was lost first from 2,4-DCP and that the 4-CP product was fairly stable.

Gibson and Suflita (4) detected phenol formation in 4-CP degradation experiments with a starting concentrations of 300 to 500 nmol/l. At similar concentrations, we have also observed the intermediate formation of phenol (unpublished results). In using an initial concentration of 125 µmol/l, however, we were not able to detect phenol as an intermediate product in this study. Also were we able to detect benzoate, which has been shown by Knoll and Winter (9) to be an intermediate in phenol degradation under a H_2/CO_2 atmosphere. These results indicate that the 4-CP dechlorination is the rate limiting step in the anaerobic biodegradation of 125 µmol/l 2,4-DCP in both the SCNS and CTP sediments and that higher concentrations of 2,4-DCP may inhibit the degradation of intermediate metabolites such as phenol.

Sulfate inhibition of methanogenic cultures actively dechlorinating chlorinated phenols has been reported by Gibson and Suflita (4). Unlike our results, they found that reductive dechlorination was completely inhibited over the 3 months of their investigation, which used initial sulfate concentrations of ≈ 0.40% and incubations at ≈ 24°C (room temperature). The inhibition was not irreversible because dechlorinating activity was restored after the sulfate had been depleted by the addition of excess acetate to stimulate dissimilatory sulfate reduction. Interestingly, the dechlorinating activity appeared to be sensitive to low concentrations of sulfate because, when acetate and sulfate were added in equal molar amounts, dechlorinating activity was not recovered. We, on the other hand, observed dechlorinating activity at sulfate concentrations that ranged between 0.08 and 0.25% and were inhibitory of methane formation and King (8) has observed that, in anaerobic marine sediments historically exposed to 2,4-dibromophenol, sulfate reducing communities could reductively dechlorinate 2,4-DCP with a half life of approximately 3 days. Apparently reductive dechlorination can occur under sulfate reducing conditions; however, the rate of the reaction will depend on the characteristics and previous exposure of the site.

Storing sediment samples before use appeared to affect adaptation more than the rate of dechlorination. For example, adaptation periods, determined over the temperature range of 5 to 25°C decreased from a range of 140 to 8 days for the fresh sediments to a range of 270 to 12 days for the sediments stored for 2 months at 12°C. The corresponding 2,4-DCP transformation rates were not significantly affected. Therefore the use of stored samples could lead to erroneous estimates of the adaptation period.

One possible reason for this storage effect on adaptation may be that organisms key to the dechlorinating process may need to reach certain concentrations before detectable transformation of 2,4-DCP can take place. Thus, storing sediment at 12°C may have reduced the population of critical organisms and consequently lengthened the time for the organisms to grow to the appropriate concentration. The growth of initially low concentrations of active organisms has accounted for the adaptation periods observed in the degradation of p-nitrophenol in lake water and sewage sludge (15), and in water/sediment systems from salt marsh and river environments (20). Alternatively, the effects could have resulted from increases in hydrogen concentrations or from decreases in intermediate carbon or energy sources.

Temperature effects are generally adequately described mathematically by the Arrhenius equation. This equation would be the preferred method for incorporating the effects of temperature into complex mathematical models used to predict the fate of organic compounds released to the environment. The effects of temperature can be extrapolated from one environment to another through the Arrhenius energy (EA) of the reaction. The EA can be determined by plotting the logarithm of the transformation rate versus the reciprocal temperature, measured in degrees Kelvin, and then the determining the slope of the linear portion of the data by linear least square analysis. Results can safely be extrapolated over the temperature range defined by the linearity of the Arrhenius plot. In this study we found that anaerobic microbial dechlorination of 2,4-DCP occurred from 5 to 52°C; however, the data were linear over only a

small portion, 15 to 35°C, of the temperature range. Above and below this linear range the temperature dependence varied markedly. Although this range appears limited, it does indicate the temperatures over which microorganisms will significantly influence the fate of chlorinated aromatic compounds in anaerobic environments and does cover a significant fraction of environments where these microorganisms would be active. Furthermore, it indicates that laboratory tests could be performed at the faster rates (e.g. at 28°C) and then extrapolated to environmental temperatures (e.g. at 20°C).

Unlike a chemical reaction where temperature may only affect the reaction, in a microbiologically mediated process, temperature affects both the composition of the active community and the enzyme-catalyzed reaction. The effects of population become even more pronounced when more than one organism, as in the case of reductive dechlorination (13), may be required for activity and different organisms may catalyze the rate determining step at different temperatures. In this study, the major peaks of activity observed above and below 40°C probably resulted from the contributions of thermophilic and mesophilic organisms, respectively. Interestingly, because thermophilic activity was only observed in half of the samples from the SCNC sediment, the key thermophilic organisms were initially present at significantly lower numbers. The smaller peaks at 30 and 35°C may have resulted from changes in the rate limiting organisms within the mesophilic community.

The variability in the correlations between the effects of temperature on dechlorination and methane formation, however, makes it difficult to assign the temperature effects to any specitic class of organisms. For example, the correlation between 2,4-DCP transformation and methane formation observed in the SCNC sediment could indicate that a close relationship exists between reductive dechlorination and methanogenic activity and that methanogenic activity may be limiting. Results with the CTP sediment, however, could indicate that a looser relationship exists between reductive dechlorination and methanogenic activity and that reductive dechlorination is the rate limiting process in this case. Apparently, in anaerobic freshwater systems a methanogenic environment is required for the dechlorination reaction to occur; however, it is premature to suggest whether the two reactions are closely coupled.

REFERENCES

1. Boyd, S. A. and D. R. Shelton. 1984. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ. Microbiol. 47:272-277.

2. Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje. 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. 46:50-54.

3. DeWeerd, K. A., J. M. Suflita, T. Linkfield, J. M. Tiedje, and P. H. Pritchard. 1986. The relationship between reductive dehalogenation and other aryl substituent removal reactions catalyzed by anaerobes. FEMS Microbiol. Ecol. 38:331-339.

4. Gibson, S. A. and J. M. Suflita. 1986. Extrapolation of biodegradation results to groundwater aquifers: Reductive dehalogenation of aromatic compounds. Appl. Environ. Microbiol. 52:681-688.

5. Guthrie, M. A., E. J. Kirsch, R. F. Wukasch, and C. P. L. Grady, Jr. 1984. Pentachlorophenol biodegradation - II: anaerobic. Water Res. 18:451-461.

6. Healy, Jr., J. B. and L. Y. Young. 1987. Catechol and phenol degradation by a methanogenic population of bacteria. Appl. Environ. Microbiol. 35:216-218.

7. Healy, Jr., J. B. and L. Y. Young. 1989. Anaerobic biodegradation of eleven aromatic compounds to methane. Appl. Environ. Microbiol. 38:84-89.

8. King, G. M. 1988. Dehalogenation in marine sediments containing natural sources of halophenols. Submitted Appl. Environ. Microbiol.

9. Knoll, G. and J. Winter. 1987. Anaerobic degradation of phenol in sewage sludge. Appl. Environ. Microbiol. 25:384-391.

10. Mikesell, M. D. and S. A. Boyd. 1985. Reductive dechlorination of the herbicides 2,4-D, 2,4,5-T and pentachlorophenol in anaerobic sewage sludge. J. Environ. Qual. 14:337-340.

11. Sahm, K., M. Brunner, and S. M. Schoberth. 1986. Anaerobic degradation of halogenated aromatic compounds. Microbiol. Ecol. 12:147-153.

12. Seuferer, S. L., H. D. Braymer, and J. J. Dunn. 1979. Metabolism of diflubenzuron by soil microorganisms and mutagenicity of the metabolites. Pestic. Biochem. Physiol. 15:213-221.

13. Shelton, D. R. and J. M. Tiedje. 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. 48:840-848.

14. Smolenski, W. J. and Suflita, J. M. 1987. Biodegradation of cresol isomers in anoxic aquifers. Appl. Environ. Microbiol. 53:710-716.

15. Spain, J. C. and P. A. Van Veld. 1983. Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure time, inoculum, and chemical structure. Appl. Environ. Microbiol. 45:428-435.

16. Suflita, J. M., A. Horowitz, D. R. Shelton, and J. M. Tiedje. 1982. Dehalogenation: A novel pathway for the anaerobic biodegradation of haloaromatic compounds. Science 218:1115-1116.

17. Suflita, J. M. and G. D. Miller. 1985. Microbial metabolism of chlorophenolic compounds in ground water aquifers. Environ. Toxicol. Chem. 4:751-758.

18. Suflita, J. M., J. A. Robinson, and J. M. Tiedje. 1983. Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. Appl. Environ. Microbiol. 45:1466-1473.

19. Tiedje, J. M., S. A. Boyd, and B. Z. Fathepure. 1987. Anaerobic degradation of chlorinated aromatic hydrocarbons. In. Pierce, G. (ed.), Dev. in Ind. Microbiol.27:117-127.

20. Wiggins, B. A., S. H. Jones, and M. Alexander. 1987. Explanations of the acclimation period preceding the mineralization of organic chemicals in aquatic environments. Appl. Environ. Microbiol. 53:791-796.

21. Young, L. Y. 1984. Anaerobic degradation of aromatic compounds, p. 487-523. In D. T. Gibson (ed.) Microbial degradation of organic compounds. Marcel Dekker, Inc., New York.

Table 1. Sediment Characterization.

Site	Water		Sediment				
			pH				
	pH	Temp. (°C)	2cm	8cm	Eh (mV)	Temp. (°C)	TOC (%)
SCNC	6.3	29	6.8	6.6	<-300	25	1.3
CTP	7.5	30	6.8	6.3	<-150	28	0.8

a Redox potential of the sediment at 8 cm.
b Temperature of the sediment at 8 cm.
c Total organic carbon of sediment (% dry wt.)

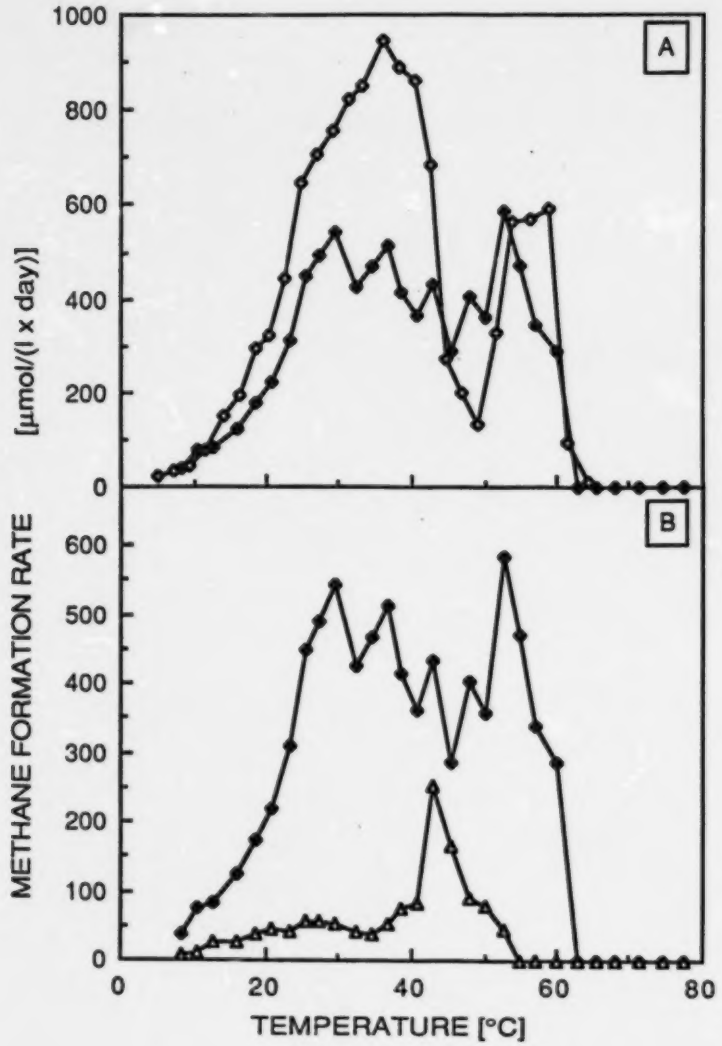

Figure 1. Effect of temperature on methane formation rates.
a) Comparison of SCNC site (◆) and CTP site (◇).
b) SCNC site in the presence (△) and absence (◆) of 0.25% sulfate.

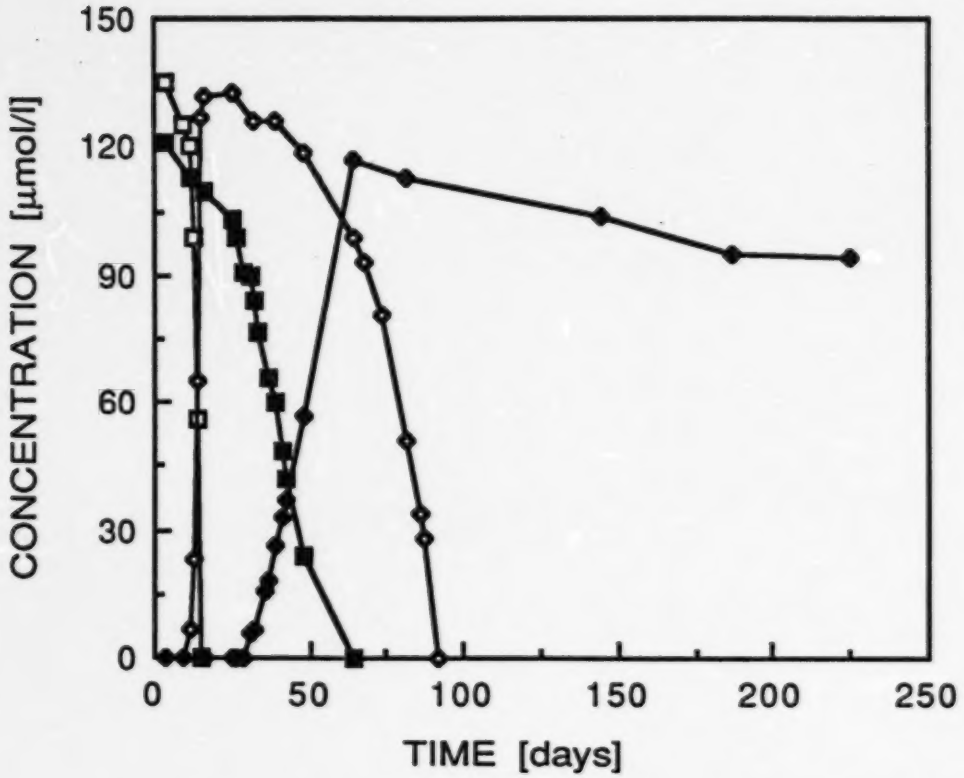

Figure 2. Reductive dechlorination of 2,4-dichlorophenol and 4-chlorophenol in SCNC sediment in the presence and absence of 0.25% sulfate. The open symbols represent the concentrations of 2,4-DCP (□) and 4-CP (◇) in the absence of sulfate and the closed symbols represent the concentrations of 2,4-DCP (■) and 4-CP (◆) in the presence of sulfate.

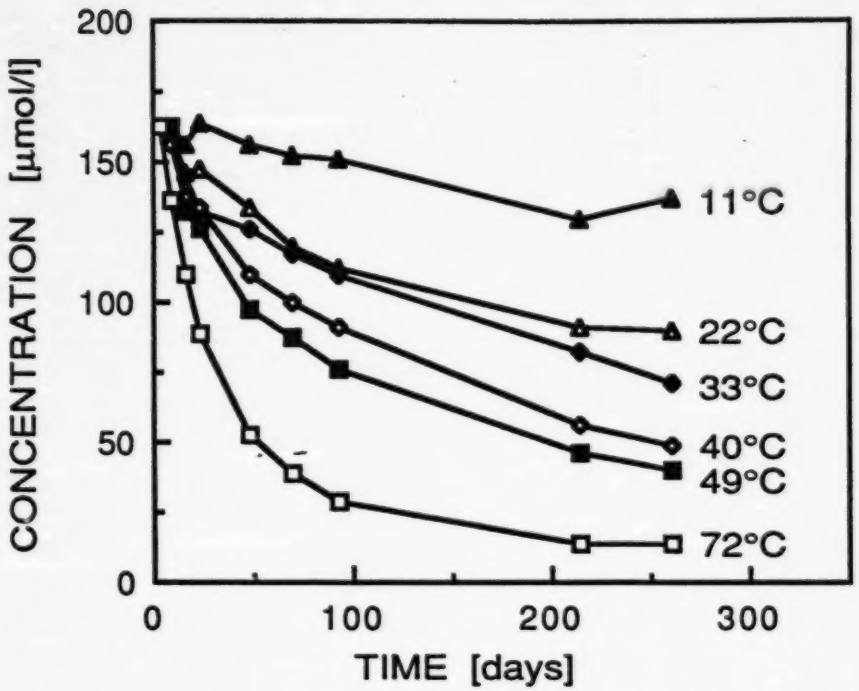

Figure 3. Abiotic losses of 2,4-DCP in autoclaved control bottles. The symbols represent the temperatures 11 (▲), 22 (△), 33 (◆), 40 (◇), 49 (■), and 72°C (□).

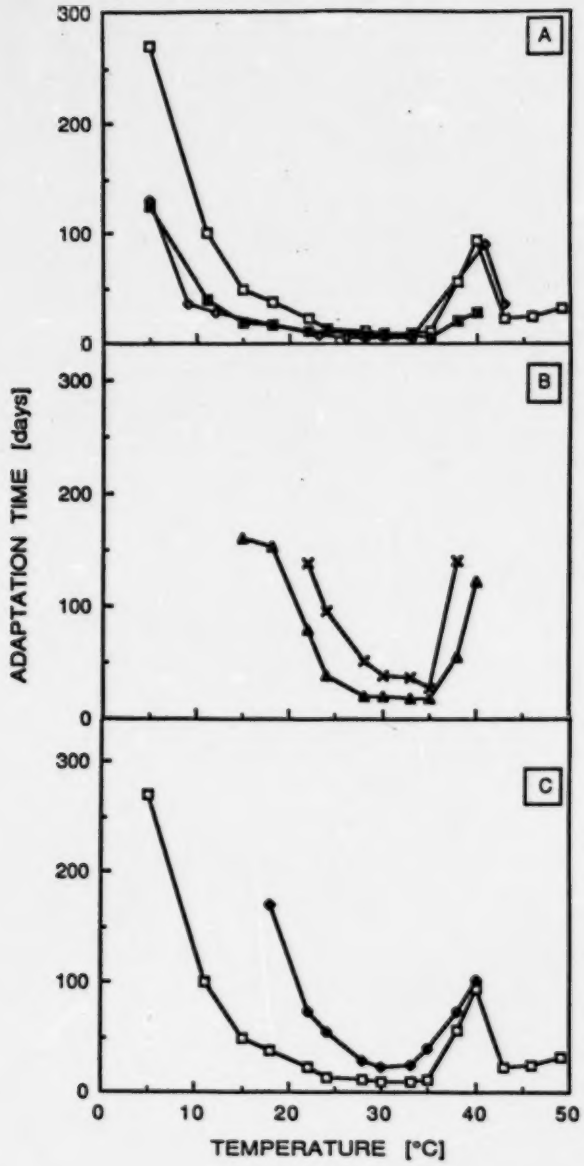

Figure 4. Effect of temperature on the adaptation of sediment to the reductive dechlorination of 2,4-DCP and 4-CP.
a) Adaptation periods for the dechlorination of 2,4-DCP in SCNC sediment (◇), stored SCNC sediment (□), and CTP sediment (■).
b) Adaptation of periods for the dechlorination of 4-CP in SCNC sediment (▲) and stored SCNC sediment (X).
c) Adaptation periods for the dechlorination of 2,4-DCP in SCNC sediment plus 0.25% sulfate (□) and stored sediment minus 0.25% sulfate (◆).

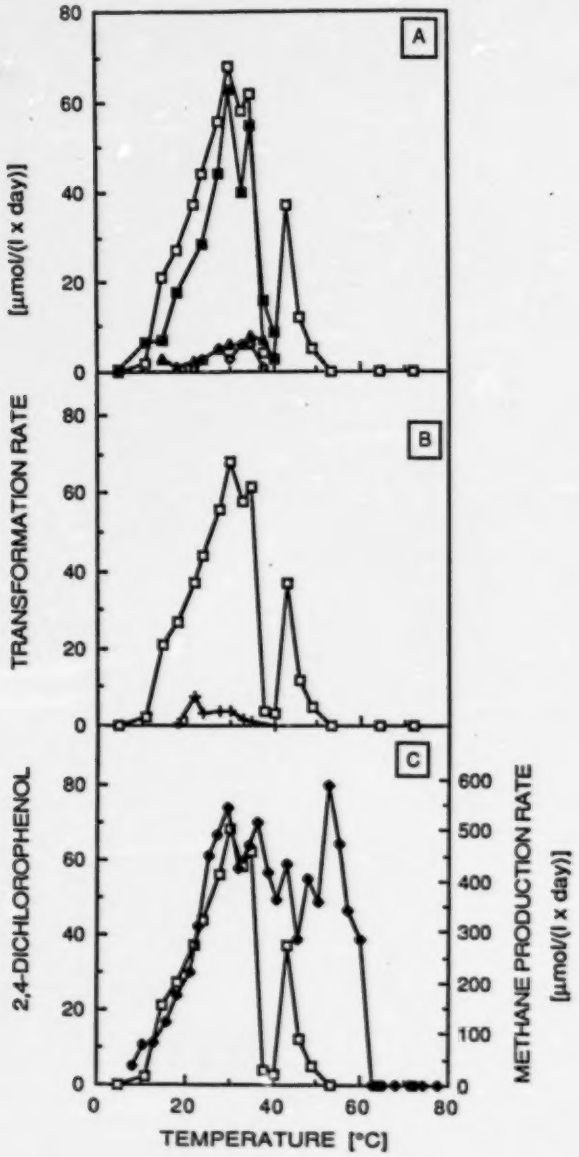

Figure 5. Effect of temperature on the rate of the reductive dechlorination of 2,4-DCP and 4-CP.

a) 2,4-DCP rates in SCNC sediment (□) and CTP sediment (■); 4-CP rates in SCNC sediments (◇) and CTP sediment (▲).

b) 2,4-DCP rates in SCNC sediment minus 0.25% sulfate (□) and SCNC sediment plus 0.25% sulfate (+).

c) Comparison of 2,4-DCP dechlorination rates (□) and methane formation rates (◆) in SCNC sediment.

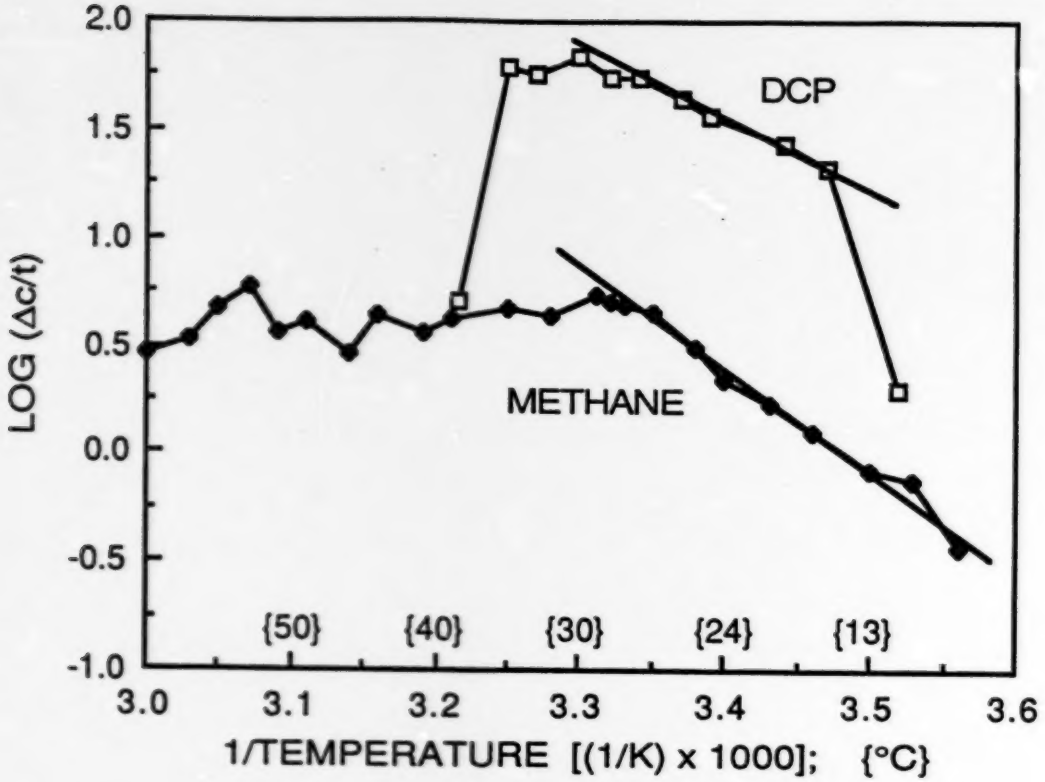

Figure 6. Arrhenius plots of the methane formation rates (◆) and the 2,4-DCP dechlorination rates (■) in sediments from the SCNC site.